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Kidney, blood, and endothelium: Developmental expression of stem cell leukemia during nephrogenesis

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Background. In vertebrates the hematopoietic and renal tissues share a common mesodermal origin. Recently, we have analyzed global gene expression during human nephrogenesis and observed up-regulation of stem cell leukemia (SCL), a transcription factor critical for hematopoietic and endothelial lineage specification. Here we characterize the expression of SCL along with its distinct 3′ hematopoietic and endothelial enhancer (SCL 3′En) during kidney development.

Methods. mRNA and protein expression of SCL were examined in developing murine and human kidneys by quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry. The activity of SCL 3′En was examined by X-galactosidase (X-gal) staining of embryonic kidneys obtained from SCL +6E5/lacZ/3′En transgenic mice and by reporter lacZ assay in various renal cell lines.

Results. We found developmental regulation of SCL mRNA with highest levels of expression in embryonic day 17 (E17) mouse kidneys and lowest in postnatal and adult kidneys. Immunostaining of human fetal kidneys demonstrated the protein predominantly in the nephrogenic cortex and particularly in mesenchymal cells and developing glomeruli. Similarly, SCL +6E5/lacZ/3′En transgenic kidneys showed prominent lacZ staining in cells resembling undifferentiated mesoderm cells in close proximity to S and comma-shaped primitive nephrons and in peritubular and glomerular vessel endothelium. The SCL 3′En was activated in the human embryonic kidney cell line (HEK 293), but not in cell lines derived from adult kidney.

Conclusion. These observations suggest a possible role for SCL in renal vasculogenesis. Undifferentiated mesenchymal cells expressing SCL during early nephrogenesis might represent putative progenitors that can simultaneously give rise to kidney, blood, and endothelium.

Key words: kidney development, SCL/TAL-1, stem cell, metanephric mesenchyme, transcription.

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The kidney is derived from the ureteric bud and the metanephrogenic mesenchyme, and these two progenitor tissues differentiate into more than 26 different cell types in the adult kidney. The ureteric bud contains the precursor of the epithelial cells of the collecting duct and the renal mesenchyme contains precursors of all the epithelia of the rest of the nephron [1]. Recent in vitro [2] and in vivo [3, 4] data suggest that cells residing in the metanephric mesenchyme are pluripotent and can differentiate, in addition to renal epithelia, into nonrenal derivatives especially of mesodermal origin, including cartilage, bone, myofibroblasts, and also blood and blood vessels.

The stem cell leukemia (SCL) gene (also known as TAL-1) encodes a basic helix-loop-helix (bHLH) protein that is commonly activated in T-cell leukemia [5]. SCL deficient mice die in utero between E9.5-10.5 with no evidence of blood formation [6, 7]. In knockout mice, yolk sac cultures confirm the absence of hemopoietic progenitors, while chimera analysis shows that $SCL^{-/-}$ ES cells are unable to contribute to any hemopoietic lineage [8, 9]. Furthermore, examination of $SCL^{-/-}$ embryoid bodies [10] demonstrates a complete failure of expression of hemopoietic genes. Thus, SCL acts as a critical regulator of hemopoiesis with a crucial function in the formation of hemopoietic stem cells (HSCs) [11]. The observation that SCL knockout mice harbor a severe defect in the development of yolk sac blood vessels along with transgenic rescue experiments proved that SCL is required for blood vessel development [6, 7, 12]. SCL also plays an important role in the specification of endothelial precursors [13]. In zebra fish development, SCL is expressed in a population of putative hemangioblasts, which give rise to

hemopoietic and endothelial progenitors. Ectopic SCL expression greatly increased the number of hemangioblasts and also resulted in excessive blood and endothelial development at the expense of other mesodermal cell fates [14]. SCL is capable, therefore, of specifying hemangioblast development from early mesoderm.

Transcriptional regulatory elements of the SCL gene have been recently characterized in detail [15, 16]. A 3′ enhancer (termed SCL 3′En) localized 19 kb downstream of exon 1 of the SCL gene was shown to have specificity for the SCL exon 4 promoter and to display particularly striking properties. It directed SCL expression to HSCs and endothelium and their bipotential precursors, the hemangioblast, and was suggested to function as a pivotal element responsible for integrating signals necessary for lineage commitment to HSCs and blood cell formation [11].

We have recently profiled gene expression during normal human kidney development and in developing human embryonic kidney precursors that were transplanted into immunodeficient mice [17]. Similar gene expression profiles, comprised of well-known regulators of renal development and genes that previously had no recognized role, were determined for both normal and transplanted human kidneys. Among the genes that were surprisingly up-regulated throughout nephrogenesis was SCL. To begin to identify the potential role of SCL in the development of the metanephros, the direct precursor of the adult kidney, we have determined the expression of SCL and its regulatory 3′En during mouse and human kidney ontogeny.

METHODS

Tissues

Normal human 10-, 14-, and 20-week gestation kidneys were obtained following curettage of elective abortions. Studies with human embryonic kidney tissue were approved by the Helsinki Ethical Committee. Normal murine embryonic and postnatal kidneys were obtained from time-dated pregnant balb/c mice (Weizmann Institute of Science Breeding Laboratory, Rehovot, Israel). Transgenic embryonic kidneys were obtained from time-dated pregnant SCL +6E5/lacZ/3′En transgenic mice. The preparation of the SCL +6E5/lacZ/3′En transgenic mice line 2296 has been previously described by Sanchez et al [15].

Cell lines

293-T, U2OS, MDCK, NIH-3T3, transformed NIH-3T3, and COS-7 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% to 10% bovine calf serum (BCS), 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Vectors

The +6E5/lacZ/3′En vector has been described in detail by Sanchez et al [15]. Briefly, the vector contains a 2.4 kb Sau3AI/Nde1 fragment starting 6 kb downstream of SCL promoter 1a with the lacZ gene fused to it and a 3′En (a 5.5 kb BglII/BglII fragment starting 14 kb downstream of SCL promoter 1a) that is located downstream of the lacZ gene (all cloned into pGL-2 vector).

The renila luciferase and cytomegalovirus (CMV)-lacZ reporter vectors encode for renila luciferase and lacZ reporter genes, respectively, under the regulation of CMV minimal promoter.

RNA preparation

Total RNA was isolated from pooled mouse embryonic kidneys at gestational day 13, 14, 15, and 17, postnatal 1 day and 2 weeks, and 90-day-old adult kidneys ($N = 3$ to 5 kidneys in each time point). Dissected tissues were immediately frozen in liquid nitrogen before RNA isolation. Total RNA was purified by using Tri-reagent isolation kits (Molecular Research Center, Cincinnati, OH, USA), according to the manufacturer's protocol.

cDNA array hybridization

We used the human 1.2 expression arrays (broad-coverage arrays) of human cDNAs spotted on a nylon membrane, which contain 1176 cDNAs (Atlas Filter Arrays) (Clontech Laboratories, Palo Alto, CA, USA), as previously demonstrated [17]. The filters also include housekeeping control cDNAs. Following RNA isolation, total RNAs were treated with Dnase I according to the manufacturer's protocol and used for cDNA synthesis. A 3 μL mix containing 5 μg of total RNA and 1 μL of 10 × commercial dialysis solution (CDS) primer mix (specific for each filter array, provided by manufacturer) (Clontech Laboratories) was incubated at 70°C for 2 minutes followed by incubation at 48°C for 2 minutes. To this mix, 8 μL of master mix [containing 2 μL 5 × reaction buffer, 1 μL 10 × desoxynucleoside triphosphate (dNTP) mix, 3.5 μL [$^{-32}$P] desoxyadenosine triphosphate (dATP) (3000 Ci/mmol, 10 mCi/mL) (Amersham/Pharmacia Biotech, Buckinghamshire, UK), 0.5 μL 100 mmol/L dithiothreitol (DTT), and 1 μL Moloney murine leukemia virus (MMLV) reverse transcriptase (50 U/μL)] were added, mixed, and incubated for 25 minutes at 48°C. The reaction was terminated by adding 1 μL of 10 × termination mix at room temperature. The radioactively labeled cDNA mix was fractionated on a Chroma Spin-200 column (Clontech Laboratories) and fractions that comprise the first peak of radioactivity were pooled for each cDNA synthesis reaction. In each set of hybridization, equal counts were taken for control and experimental labeled cDNA probes. The labeled cDNA probe was then mixed with 1/10 volume of 10 ×

denaturing solution [1 mol/L NaOH, 10 mmol/L ethylenediaminetetraacetic acid (EDTA)] and incubated at 68°C for 20 minutes followed by the addition of 5 μL (1 μg/μL) of cot-1 DNA and an equal volume of 2 × neutralizing solution (1 mol/L NaH_2PO_4, pH 7.0), and incubated at 68°C for 10 minutes. Denatured, labeled cDNA was then added to 5 mL of ExpressHyb solution (Clontech Laboratories) with 1 mg of sheared salmon sperm DNA (Sigma, St. Louis, MO, USA) and mixed. This hybridization solution was added to the Atlas cDNA Expression Array membrane, which was prehybridized in 10 mL of ExpressHyb hybridization solution at 68°C for 1 hour. Hybridization preceded overnight at 68°C in a roller bottle. Membranes were washed once with prewarmed 2 × standard sodium citrate (SSC)/1% sodium dodecyl sulfate (SDS) for 30 minutes and once or twice with 0.5 × SSC/0.5% SDS for 30 minutes at 68°C with constant agitation. The membranes were exposed to Fuji x-ray films at 70°C with intensifying screens.

cDNA generation and real-time quantitative polymerase chain reaction

cDNA was generated using Reverse-It 1st Strand Synthesis Kit (AB Gene, catalog no. AB-0789/B; Epsom, UK) with random decamers, following manufacturer's instructions.

Real-time quantitative polymerase chain reaction (RQ-PCR) assays were developed to determine the level of expression of SCL. SCL expression was normalized with β-actin expression as internal standard. Forward and reverse primers were designed in exon-intron junctions in order to eliminate DNA contamination. Primers (Eisenberg Bros., Ltd., Givat Shmuel, Israel) were designed according to primer express software guidelines (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA): SCL forward primer, 5′-CATGTTCACCAAC AACAACCG-3′; SCL reverse primer, 5′-GGTGTGA GGACCATCAGAAATCTC-3′; β-actin forward primer, 5′-TCCTGGCCTCACTGTCCAC-3′; and β-actin reverse primer, 5′-GTCCGCCTAGAAGCACTTGC-3′. The real-time PCR reactions were performed in a total volume of 20 μL containing 500 nmol/L of each forward and reverse primers and Master Mix for Syber Green (Applied Biosystems, Foster City, CA, USA) with cDNA equivalent to 100 ng of RNA (for each time point) using Applied Biosystems 7900HT prism real-time PCR instrument (TaqMan) (Perkin-Elmer/Applied Biosystems). PCR amplification included a first step of 10 minutes at 95°C followed by 40 cycles of amplification (95°C for 15 seconds and 60°C for 60 seconds). Standard curves were generated using RNA extracted from mouse spleen (which expresses SCL) diluted in four log steps. Ratio was calculated by dividing each gene expression with that of an internal standard in each sample. These ratios were compared between the different time points (average ± SEM). Three independent experiments were performed for each time point analyzed.

Immunohistology

Immunohistochemistry for SCL was done using a monoclonal anti-SCL/TAL-1 antibody (2TL242), kindly provided by Dr. Karen Pulford, Oxford, United Kingdom. The 2TL242 antibody raised against recombinant SCL/TAL-1 protein has been previously characterized for immunohistochemistry and was shown to specifically recognize TAL-1 polypeptides of molecular weight 39 and 41 kD (full length) [18, 19]. Briefly, sections of 5 μm were mounted on superfrost/plus glass (Menzel, Glazer, Braunschweig, Germany) and processed by the labeled (streptavidin) avidin-biotin (LAB-SA) method using a histostain plus kit (Zymed, San Francisco, CA, USA). Heat-induced antigen retrieval was performed by controlled microwave treatment using an H2800 model processor (Energy Bean Sciences, Inc., Agawan, MA, USA) in 10 mmol/L citrate buffer, pH 6.0, for 10 minutes at 97°C. The sections were treated with 3% H_2O_2 for 5 minutes. Consecutive sections were incubated for 1 hour with the 2TL242 antibody. Negative control incubations were performed by substituting nonimmune serum for the primary antibody. Biotinylated second antibody was applied for 10 minutes followed by incubation with horseradish peroxidase–conjugated streptavidin (HRP-SA) for 10 minutes. Following each incubation, the slides were washed thoroughly with Optimax wash buffer (Biogenex, San Ramon, CA, USA). The immunoreaction was visualized by an HRP-based chromogen/substrate system, including diaminobenzidine (DAB) (brown) chromogen (liquid DAB substrate kit) (Zymed). The sections were then counterstained with Mayer's hematoxylin, dehydrated, and mounted for microscopic examination.

β-Galactosidase detection

X-galactosidase (X-gal) staining was performed as previously described [15]. Embryonic kidneys were incubated overnight in staining solution. β-galactosidase (β-gal) expression was visualized in less than 1 hour. Prior to sectioning, the embryos were dehydrated with increasing concentrations of ethanol and mounted in paraffin wax. Six to 10 mm sections were counterstained with neutral red.

Transient transfection and reporter assays

The 293-T and U2OS cell lines were transfected using calcium phosphate method. The NIH-3T3, transformed NIH-3T3, COS-7, and Madin-Darby canine kidney

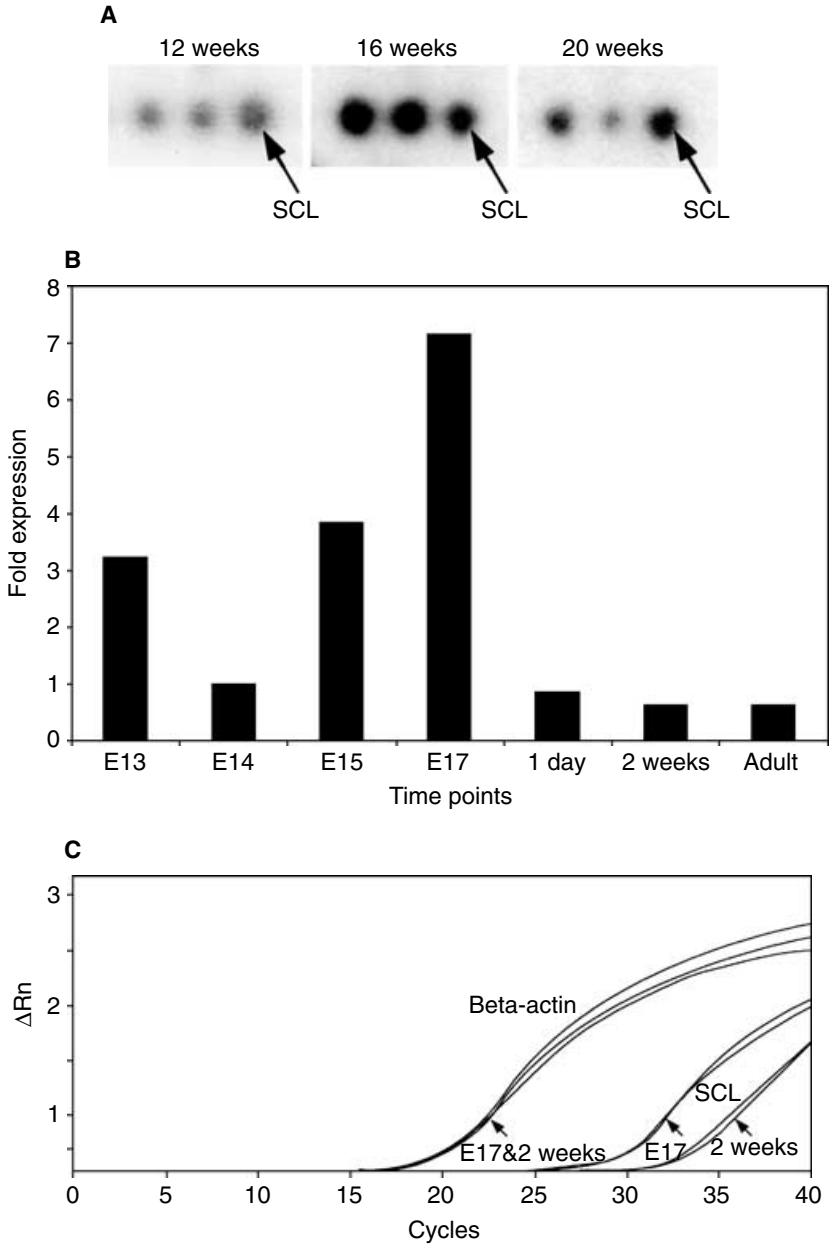

Fig. 1. Stem cell leukemia (SCL) gene expression in the developing kidney. (*A*) Hybridization to cDNA of SCL on broad-coverage human expression arrays when RNA used for probes was derived from normal human 12-, 16-, and 20-week-old gestation kidneys (arrows point to SCL). (*B*) Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of SCL mRNA levels in the developing mouse kidney. Shown is a representative example of three independent experiments. Total RNA was isolated from pooled mouse embryonic (E) kidneys ($N = 3$ to 5 kidneys in each time point). (*C*) A representative amplification plot of the real-time RT-PCR reaction shows the SCL expression differential between embryonic day 17 and postnatal 2 weeks kidneys as compared to equal β-actin expression in both time points. Fold expression denotes the relative expression of SCL compared with adult kidney.

(MDCK) were transfected using lipofectamine plus reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocols. Briefly, cells were plated in 24-well plates the day before transfection. Total DNA and lipofectamine plus reagent amounts were according to cell type and manufacturer's protocol. The transfection was done in 250 μL volume of antibiotic-free media and finally 1 mL media was added to the cells 3 hours later. For the reporter assays, cells were harvested 36 to 48 hours after transfection and lysed with Promega reporter lysis buffer (Promega, Madison, WI, USA). LacZ readings were normalized according to the renila luciferase vector readings and the CMV-lacZ reporter vector served as a positive control in each cell line.

RESULTS

SCL mRNA expression in developing kidneys

A representative section from the atlas human 1.2 array (Clontech Laboratories), demonstrating positive hybridization to SCL cDNA filter when RNA used for probes was derived from 12-, 16-, and 20-week-old human gestation kidneys is shown in Figure 1A. If SCL plays a role in the regulation of kidney development, it is expected that SCL expression would be developmentally regulated. To test this hypothesis, we quantitatively analyzed SCL transcripts in developing murine kidneys of different gestational ages. As shown in Figure 1B and C, SCL mRNA is significantly higher in the embryonic

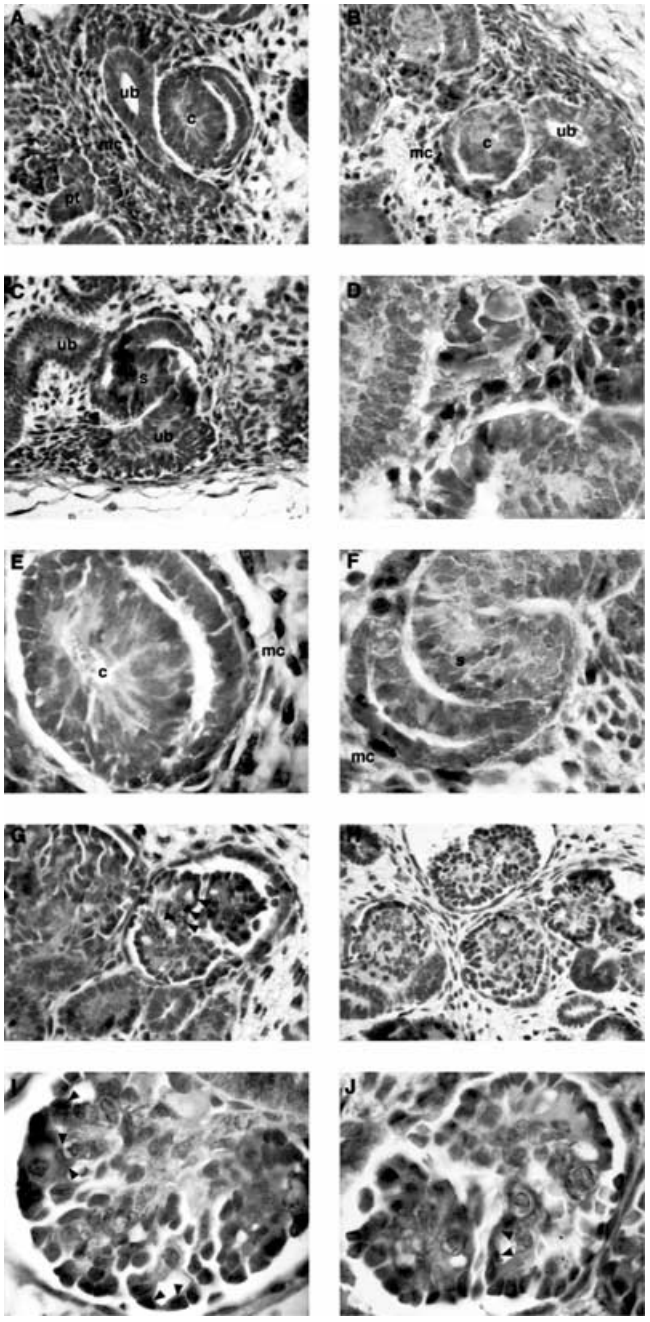

Fig. 2. Immunolocalization of stem cell leukemia (SCL) in developing human kidneys. (*A* to *F*) Different sections of a 10-week-old human gestation kidney nephrogenic cortex. (A and B) Low magnification (original, ×40) and (D to F) and higher magnification (original, ×100) clearly show in all instances positive staining in metanephric mesenchymal cells (mc) and in few cells of the comma (c) and S (s)-shaped primitive nephrons. (C) Control section of the nephrogenic cortex without the 2TL242 antibody demonstrates minimal background staining with no staining in mesenchymal cells. (*G* to *J*) Sections of more mature fetal kidneys showing in (G) low magnification (original, ×40), positive staining for SCL in developing glomeruli (arrowheads), which is shown in high magnification (original, ×100) in (I and J) to be localized to developing glomerular capillaries (arrowheads) and intracapillary erythrocytes (J, arrow). (H) Control section of developing glomeruli without the 2TL242 antibody demonstrates minimal background staining.

kidneys and rapidly falls after birth, with the lowest levels in the adult kidney. SCL mRNA levels are highest in the embryonic day 17 (E17) kidney, the time of appearance of well-vascularized glomeruli. Interestingly we also observed a slight but consistent reduction in SCL mRNA levels during transition from early nephrogenesis to a more mature stage (E13 to E14) (Fig. 1B). Thus, SCL is expressed during embryonic nephrogenesis in a developmentally regulated manner.

Immunolocalization of SCL in the developing kidney

SCL-expressing cells were mapped in serial sections of the developing human kidney. As shown in low magnification (Fig. 2A and B), SCL is localized to the nephrogenic cortex of a 10-week-old human gestation kidney, particularly in renal mesenchyme, but not in ureteric buds, developing comma-shaped nephron, or more mature tubules. Several fields of the nephrogenic cortex shown in higher magnification in Figure 2D to F clearly demonstrate positive staining of clusters of mesenchymal cells, some of which are in close proximity to comma and S-shaped bodies (Fig. 2E and F, respectively). Staining is also seen in few cells in the early nephron precursors. Immunohystochemical analysis of more mature fetal kidneys (14- and 20-week-old human gestation kidneys) showed that developing glomeruli, especially capillary stage, also stain positive for SCL (Fig. 2G, arrowheads). Higher magnification demonstrated specific staining of glomerular endothelial cells, based on their morphology and localization within the glomerulus, and of red blood cells (Fig. 2I and J). Maturing tubules (proximal, distal, and collecting) were all negative for SCL (data not shown). The control sections of nephrogenic cortex and developing glomeruli shown in Figure 2C and H, respectively, demonstrate minimal background staining, confirming the specificity of the immunohystochemical results. Thus, the SCL protein is detected throughout human nephrogenesis with a specific immunohystochemical staining pattern of cells of the nephrogenic cortex and glomeruli.

SCL 3′En expression in the developing kidney

Because SCL expression is developmentally regulated in the kidney, SCL 3′En activity was examined to determine whether there is a correlation between the expression of SCL and the activity of its major hemoangioblastic regulatory element. We assessed the activity of this enhancer in embryonic kidneys obtained from mice transgenic for the +6E5/lacZ/3′En construct. Whole-mount X-gal staining was used to assess transgene expression in E13, E14, E16, and adult kidneys. As shown in Figure 3A and B in low magnification, lacZ is highly expressed in the E13 kidneys, particularly in cells of the renal mesenchyme residing in close proximity to the comma- and S-shaped primitive nephrons (Fig. 3C and D), whereas

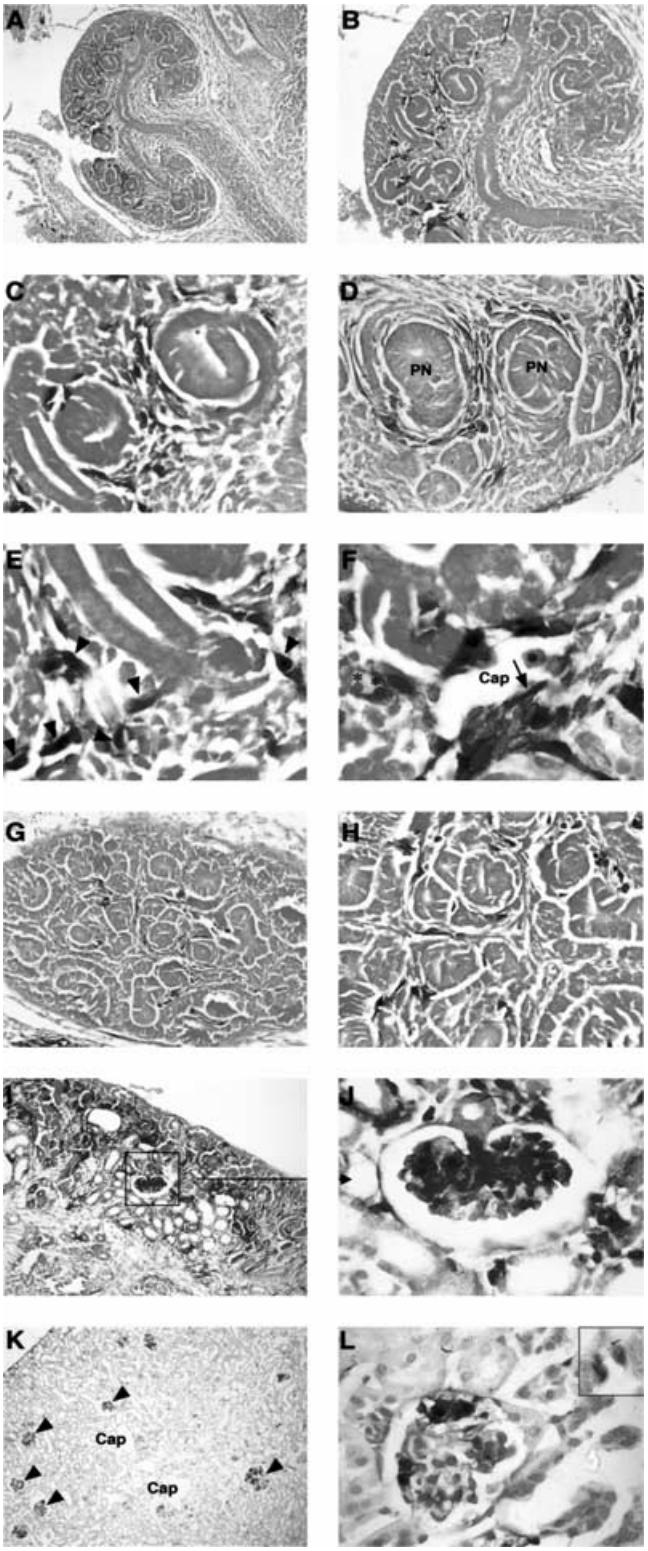

Fig. 3. Expression of +6E5/*lacZ*/3′En transgene in developing mouse embryonic kidneys. X-galactosidase (X-gal) staining of embryonic day 13 (E13) (*A* to *F*), E14 (*G* and *H*), E16 *(I* and *J*), and adult kidneys (*K* and *L*). (A and B) Low magnification images (original, ×10 and ×20, respectively) show abundant lacZ expression in the nephrogenic cortex. (C and D) Positive X-gal staining in cells of the mesenchyme surround comma and S-shaped primitive nephrons (PN). (E and F) High magnification images (original, ×100) show prominent lacZ staining mesodermal cells (E, arrowheads) and endothelial cells organized in capillaries (Cap) (F, arrow). Note negative staining of intracapillary erythrocytes is marked with asterisks. (G and H) Reduced lacZ expression in E14 kidneys is shown in low (original, ×20) and higher magnification (original, ×40), respectively, whereas E16 kidneys demonstrate prominent lacZ staining (original, ×20) (I), especially in developing glomeruli (original, ×100) (J). (K and L) X-gal staining of adult kidneys shows overall reduced lacZ expression that localizes to glomeruli [low magnification in (K) (original, ×10, arrowheads) and higher magnification in (L) (original, ×100)], peritubular capillaries (K, Cap), and scattered interstitial cells (L, right upper inset).

epithelial structures were all negative. Furthermore, higher magnification revealed prominent lacZ staining in cells resembling less-differentiated mesoderm cells (Fig. 3E, arrowheads) and in endothelial cells organized in capillaries (Fig. 3F, arrow). There was relatively less expression of lacZ in the E14 kidneys (Fig. 3G and H), whereas in E16 kidneys prominent X-gal staining was observed in developing glomeruli, which start to appear in E15 kidneys (Fig. 3I and J). In adult kidneys, although significantly less abundant, expression of lacZ was still observed in glomeruli, peritubular capillaries, and interestingly also in scattered interstitial cells (Fig. 3K and L). Therefore, the robust activity of the regulatory 3′En in the developing kidney is closely related to that of SCL protein and mRNA.

In vitro analysis of expression of SCL 3′En

In addition to the delineation of the in vivo expression pattern of the SCL 3′En, we examined its activity by luciferase reporter assays in various cell lines. For these experiments we used the following cell lines: COS-7, derived from an adult African green monkey kidney; MDCK, an epithelial cell line derived from adult cocker spaniel kidney; and 293-T, derived from human embryonic kidney and transformed by the sv-40 T antigen. For controls, we used nonrenal cell lines: U2OS, osteosarcoma-derived cell line, and NIH-3T3, human embryonic fibroblast cell line, as well as a sv-40 T antigen transformed NIH-3T3 cell line to rule out nonspecific activation in a transformed cell line. Interestingly, the +6e5/lacZ/3′En vector directed the expression of lacZ only in the human embryonic kidney cell line 293-T but not in any of the other renal or control cell lines (Fig. 4).

DISCUSSION

Recent studies suggest that there may be unexpected plasticity in the relationship between metanephric renal mesenchymal cells and other professional cell fates apart from epithelial cells of the nephron. For instance, Almeida-Porada et al [3] showed that human metanephric mesenchymal cells engraft and demonstrate

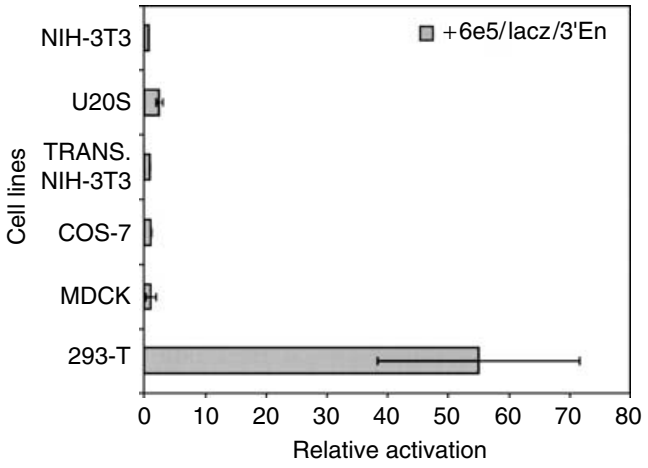

Fig. 4. Reporter assays for the determination of stem cell leukemia (SCL) regulatory element activity (SCL 3′ enhancer) in various renal and nonrenal cell lines. β-galactosidase reading demonstrates that +6e5/lacz/3 enhancer vector directed the expression of lacZ only in the human embryonic kidney cell line 293-T, but not in any of the other cell lines analyzed (transient transfection and reporter assay are detailed in the **Methods** section).

differentiation into CD34+ HSCs after transplantation into sheep fetuses. The transplanted human renal cells produced multilineage hematopoietic engraftment. Oliver et al [2] demonstrated that a metanephric mesenchymal cell line (7.1.1 cells) and primary cultures of metanephric mesenchymal cells express vascular endothelial growth factor receptor 2 and Tie-2 and suggested that these epithelia-generating cells may also differentiate into endothelial cells. Similarly, we have previously shown that very early porcine kidney precursors can give rise to organized blood vessels after transplantation into immunodeficient mice [4]. It is therefore of great interest to determine the expression of a gene, which has been shown to be capable of integrating bipotential information needed for blood and endothelial development, during kidney ontogeny. Moreover, if cells that posses dormant hemogenic and vasculogenic potential reside in the adult kidney and could be activated in manner analogous to embryonic induction [20], they might be an important factor in regeneration after injury.

Quantitative gene expression measurements, immunohistology and in vitro and in vivo regulatory element analyses combine together to suggest a role for SCL in kidney development. Real-time reverse transcription (RT)-PCR of total kidney RNA demonstrates that SCL mRNA is developmentally regulated in the murine kidney with highest levels in late nephrogenesis and a significant decrease in the postnatal period. Importantly, prominent transcript levels are already noted early in nephrogenesis with a slight reduction prior to induction of peak mRNA levels. These temporal changes correlate with the extent of X-gal staining in developing mouse kidneys transgenic for the distinct hematopoietic and endothelial 3′En of SCL and with its cell-specific expression pattern. Thus, in early mouse nephrogenesis, prior to the induction of glomeruli (E13), there is abundant expression of SCL 3′En, which is restricted predominantly to clusters of mesoderm cells of the nephrogenic mesenchyme in proximity to primitive nephrons and also to small capillaries. The in vitro demonstration that the SCL 3′En is activated exclusively in an embryonic kidney-derived cell line, which has been recently characterized and was shown to express mesenchymal cell markers, is consistent with this finding [21].

Our data therefore demonstrate that the +6E5/lacZ/3′En construct directed lacZ expression to presumptive mesodermal progenitors at E13 mouse kidneys. Later on, with the advent of glomerulogenesis (E16), extensive transgene expression is found predominantly in the developing glomeruli. This cell-specific pattern of expression was very similar to that displayed by immunostaining for SCL protein in developing human kidneys. Respectively, in the embryonic (10 weeks) and fetal (20 weeks) human kidneys, SCL protein mainly localizes to cells of the metanephric mesenchyme and to developing glomeruli.

Our findings of SCL and SCL 3′En expression in peritubular and glomerular blood vessels are in good agreement with several experiments which showed both to be expressed in endothelial cells during embryogenesis and adult tissues [18, 22, 23]. Accordingly, we could also demonstrate continued lacZ expression in mature glomeruli and peritubular capillaries of adult transgenic kidneys, and even in some interstitial cells.

The pattern of SCL expression during mammalian kidney development indicates that SCL may be important in the early differentiation and growth of specific cells within the metanephric mesenchyme. This hypothesis is supported by several lines of evidence. First, early studies in cell lines and primary hemopoietic cells support a role for SCL in the control of proliferation, self-renewal, terminal differentiation, and survival [24, 25]. Second, SCL is expressed in early mesenchymal progenitors of the zebra fish pronephros and when overexpressed specifies hemangioblast development [14]. Third, GATA and Ets transcription factors, which regulate the SCL stem cell enhancer and coactivate SCL transcription [26], were also shown to be expressed in the mammalian metanephros [27, 28].

It has been recently demonstrated that rather than ingrowth of vessels into the developing kidney, endothelial precursors residing in the metanephrogenic mesenchyme itself give rise, at least in part, to glomerular capillaries and microcirculation of the developed kidney [1, 29]. Whether SCL and its regulatory subunits function in the specification of a population of such precursor cells in the embryonic renal mesenchyme and are therefore important to glomeular development remains to be explored.

Moreover, in the light of previous evidence that SCL is expressed in the hemangioblast [14] and the suggestion that in early embryogenesis the SCL 3′En directs expression to mesodermal cells as they are activating the transcriptional programs necessary for hematopoietic and endothelial differentiation [15, 16], metanephric SCL-expressing cells may represent less committed renal precursor cells that are able to generate blood and/or endothelium, in addition to epithelia.

CONCLUSION

The present study demonstrates that SCL gene and protein expression and the activity of the SCL stem cell enhancer are developmentally regulated in the mammalian kidney. The early and specific expression of SCL suggests a role for this hematopoietic and endothelial master regulator in the differentiation and growth of the mammalian kidney.

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REFERENCES

1. Al-Awqati Q, Oliver JA: Stem cells in the kidney. *Kidney Int* 61:387–395, 2002
2. Oliver JA, Barasch J, Yang J, Herzlinger D, *et al*: Metanephric mesenchyme contains embryonic renal stem cells. *Am J Physiol Renal Physiol* 283:F799–F809, 2002
3. Almeida-Porada G, El Shabrawy D, Porada C, *et al*: Differentiative potential of human metanephric mesenchymal cells. *Exp Hematol* 30:1454–1462, 2002
4. Dekel B, Burakova T, Arditti FD, *et al*: Human and porcine early kidney precursors as a new source for transplantation: *Nat Med* 9:53–60, 2003
5. Begley CG, Green AR: The SCL gene: From case report to critical hematopoietic regulator. *Blood* 93:2760–2770, 1999
6. Robb L, Lyons I, Li R, *et al*: Absence of yolk sac hematopoiesis from mice with a targeted disruption of the *scl* gene. *Proc Natl Acad Sci USA* 92:7075–7079, 1995
7. Shivdasani RA, Mayer EL, Orkin SH: Absence of blood formation in mice lacking the T-cell leukemia oncoprotein tal-1/SCL. *Nature* 373:432–434, 1995
8. Robb L, Elwood NJ, Elefanty AG, *et al*: The SCL gene product is required for the generation of all hematopoietic lineages in the adult mouse. *EMBO J* 15:4123–4129, 1996
9. Porcher C, Swat W, Rockwell K, *et al*: The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell* 86:47–57, 1996
10. Elefanty AG, Robb L, Birner R, *et al*: Hematopoietic-specific genes are not induced during in vitro differentiation of scl-null embryonic stem cells. *Blood* 90:1435–1447, 1997
11. Bloor AJ, Sanchez MJ, Green AR, *et al*: The role of the stem cell leukemia (SCL) gene in hematopoietic and endothelial lineage specification. *J Hematother Stem Cell Res* 11:195–206, 2002
12. Visvader JE, Fujiwara Y, Orkin SH: Unsuspected role for the T-cell leukemia protein SCL/tal-1 in vascular development. *Genes Dev* 12:473–479, 1998
13. Liao EC, Paw BH, Oates AC, *et al*: SCL/Tal-1 transcription factor acts downstream of cloche to specify hematopoietic and vascular progenitors in zebrafish. *Genes Dev* 12:621–626, 1998
14. Gering M, Rodaway ARF, Gottgens B, *et al*: The SCL gene specifies haemangioblast development from early mesoderm. *EMBO J* 17:4029–4045, 1998
15. Sanchez M, Gottgens B, Sinclair AM, *et al*: An SCL 3′ enhancer targets developing endothelium together with embryonic and adult haematopoietic progenitors. *Development* 126:3891–3904, 1999
16. Sanchez MJ, Bockamp EO, Miller J, *et al*: Selective rescue of early haematopoietic progenitors in SCL(−/−) mice by expressing SCLnder the control of a stem cell enhancer. *Development* 128:4815–4827, 2001
17. Dekel B, Amariglio N, Kaminski N, *et al*: Engraftment and differentiation of human metanephroi into functional mature nephrons after transplantation into mice is accompanied by a profile of gene expression similar to normal human kidney development. *J Am Soc Nephrol* 13:977–990, 2002
18. Pulford K, Lecointe N, Leroy-Viard K, *et al*: Expression of TAL-1 proteins in human tissues. *Blood* 85:675–684, 1995
19. Chetty R, Pulford K, Jones M, *et al*: An immunohistochemical study of TAL-1 protein expression in leukaemias and lymphomas with a novel monoclonal antibody, 2TL 242. *J Pathol* 178:311–315, 1996
20. Polesskaya A, Seale P, Rudnicki MA: Wnt signaling induces the myogenic specification of resident CD45+ adult stem cells during muscle regeneration. *Cell* 113:841–852, 2003
21. Shaw G, Morse S, Ararat M, *et al*: Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. *FASEB J* 16:869–871, 2002
22. Green AR, Visvader J, Lints T, *et al*: SCL is co-expressed with GATA-1 in haemopoietic cells but is also expressed in developing brain. *Oncogene* 7:653–660, 1992
23. Kallianpur AR, Jordan JE, Brandt SJ: The SCL/TAL-1 gene is expressed in progenitors of both the hematopoietic and vascular systems during embryogenesis. *Blood* 83:1200–1208, 1994
24. Aplan PD, Nakahra K, Orkin SH, *et al*: The SCL gene product: A positive regulator of erythroid differentiation. *EMBO J* 11:4073–4081, 1992
25. Chiba T, Nagata Y, Kishi A, *et al*: Induction of erythroid-specific gene expression in lymphoid cells. *Proc Natl Acad Sci USA* 90:11593–11597, 1993
26. Gottgens B, Nastos A, Kinston S, *et al*: Establishing the transcriptional programme for blood: the SCL stem cell enhancer is regulated by a multiprotein complex containing Ets and GATA factors. *EMBO J* 21:3039–3050, 2002
27. Bell E, Lumsden A, Graham A: Expression of GATA-2 in the developing avian rhombencephalon. *Mech Dev* 84:173–176, 1999
28. Razzaque MS, Naito T, Taguchi T: Proto-oncogene Ets-1 and the kidney. *Nephron* 89:1–4, 2001
29. Woolf AS, Loughna S: Origin of glomerular capillaries: Is the verdict in? *Exp Nephrol* 6:17–21, 1998